U.S. DEPARTMENT OF COMMERCE
National Technical Information Service

PB-259 646-T

Comparative Toxicological Assessment of Dithiocarbamates

Environmental Protection Agency, Research Triangle Park, N C

1972

PB 259 646

321013

EPA-TR-337-73

COMPARATIVE TOXICOLOGICAL ASSESSMENT OF DITHIOCARBAMATES

[Sravnitel'naya toksikologicheskaya otsenka ditiokarbamatov]

E.A. Antonovich, O.V. Chernov, L.V. Samosh, L.V. Martson, M.A. Pilinskaya, L.I. Kurinny, M.Sh. Vekshtein, V.S. Martson, P.N. Balin and I.I. Khitsenko

Gigiena i Sanitariya, August, 1972, pp. 25 - 30.

Translated for APTIC by
SCITRAN (SCIENTIFIC TRANSLATION SERVICE)
SANTA BARBARA, CALIFORNIA

REPRODUCED BY
NATIONAL TECHNICAL
INFORMATION SERVICE
U.S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA. 22161

BIBLIOGRAPHIC DATA SHEET	1. Report No. EPA TR73-337	2.	3. Recipient's Accession No.
4. Title and Subtitle: Comparative toxicological assessment of dithiocarbamates			5. Report Date: 1972 6.
7. Author(s) E.A. Antonovich, O.V. Chernov, L.V. Samosh, L.V. Martson M.A. Pilinskaya, L.I. Kurinny, M.Sh. Vekshtein, et.al.			8. Performing Organization Rept. No.
9. Performing Organization Name and Address: TRANSLATED FROM RUSSIAN BY EPA			10. Project/Task/Work Unit No. 11. Contract/Grant No.
12. Sponsoring Organization Name and Address: Translation Service Office of Administration - Library Research Triangle Park, NC 27711			13. Type of Report & Period Covered 14.

15. Supplementary Notes
GIGIENA I SANITARIYA, August 1972, pp.25-30

16. Abstracts

17. Key Words and Document Analysis. 17a. Descriptors

EPAISD

17b. Identifiers/Open-Ended Terms

PESTICIDES
TRACE ELEMENTS-HEALTH EFFECTS

17c. COSATI Field/Group 06T, 06F 68E, 57Y

18. Availability Statement: THIS TRANSLATION WAS MADE FROM A NON-COPYRIGHTED TEXT.	19. Security Class (This Report) UNCLASSIFIED	21. No. of Pages 11
	20. Security Class (This Page) UNCLASSIFIED	22. Price $3.50--3.00

USCOMM-DC 40329-P71

COMPARATIVE TOXICOLOGICAL ASSESSMENT OF DITHIOCARBAMATES

E. A. Antonovich, O. V. Chernov, L. V. Samosh, L. V. Martson, M. A. Pilinskaya, L. I. Kurinny, M. Sh. Vekshtein, V. S. Martson, P. N. Balin, I. I. Khitsenko

ABSTRACT. A comparative toxicological assessment of TMTD, cirame, cineb, maneb and polycarbacine proved cirame, TMTD and maneb to be more dangerous. In comparison with maneb and cineb, cirame has a more pronounced cumulative, teratogenic, gonadotoxic, and blastogenic effect and a much higher cytogenic activity. Maneb surpasses the rest of these pesticides in embryotoxic and blastogenic activity. The extent of the mutagenic effect and the toxicity in chronic poisoning of TMTD exceeded that of cirame, cineb and maneb. All the pathological effects produced were least pronounced in the action of cineb and polycarbacine.

In recent years we have conducted toxicological-hygienic studies of dithiocarbamic acid derivatives used in agriculture and in the production of rubber. We studied the toxicity of pesticides in single and multiple administration to animals through the gastro-intestinal tract. We found their embryotoxic, gonadotoxic and blastomogenic properties and cytogenetic effect. We studied the metabolism of these substances in the organism of warm blooded animals as well as in food and several industrial crops.

Of the dimethyl and ethylene-bis-dithiocarbamic acid derivatives studied, the most toxic in single administration to the stomach were tetramethylthiuramdisulfide (TMTD) and cirame (Table 1). Their cumulative effect is also more pronounced. The coefficient of cumulation with TMTD administered

in doses of 1/10 and 1/20 LD_{50} is 2.1-1.85, cirame is 1.2-2.9. The cumulative effect of cirame increases as dosage decreases. The coefficient of cumulation of cupracine is slightly higher, 3.63. In other pesticides we investigated, cumulative properties are weak. Rats tolerate repeated administration of maneb, cineb and polycartacine in total dosages which are 10-12 times higher than LD_{50}. Prolonged (a year or more) treatment of the organism with TMTD and cirame in comparatively small amounts (1-10 mg/kg) leads to the development of chronic poisoning. Thus, when rats are given TMTD in doses from 4 to 1 mg/kg of body weight, changes in the morphological condition of the blood and the functional condition of the liver, disturbances in the enzyme system regulating oxidation-reduction processes and structural changes in organs and tissues are observed.

It has been established (Ye. A. Antonovich and S. Ye. Braginskiy; Ye. A. Antonovich and M. Sh. Vekshteyn) that dithiocarbamates accumulate in the liver, kidneys, heart, spleen, brain, stomach and intestinal walls, muscles, adrenals, pancreas, thymus and sex glands. Maximum accumulation of pesticides is noted in the endocrine system (Fig. 1 and 2).

Dimethyl dithiocarbamates in the organism form tetramethylthiourea, amine salt of dimethyl dithiocarbamic acid, carbon bisulfide and other compounds. TMDT develops during the process of cirame metabolism. During the process of transforming ethylene-bis-dithiocarbamates in warm blooded animals ethylenethiourea, carbon bisulfide, etc. are formed. Dithiocarbamates and their derivatives are excreted with urine, feces and expired air. Last of all (up to 4 weeks) TMTD is excreted from the organism. Other dithiocarbamates we studied remain in the organism from 4 to 6 days.

In experiments on white rats (20 animals per group) we studied the effect of cineb, cirame, maneb and polycarbacine on embryogenesis. Preparations were administered to pregnant animals with milk to the stomach every other day during the period of intense differentiation of organs and tissues of the embryo (from the 9-13th day to the end of pregnancy). A portion of the animals were

TABLE 1. TOXICITY OF DITHIOCARBAMATES IN SINGLE AND MULTIPLE ADMINISTRATION TO THE STOMACH

Pesticide	Kind of animal	Dose (in mg/kg)		
		Single administration	Multiple administration	
		LD_{50}	Toxic	Ineffective
TMTD	Rats	400±68 (industrial compound)	4–1	0.5
	Rats	1240 (pure compound) 1230 (industrial compound	10	1.0
Cirame	Mice	362 (pure) 337 (industrial)		
	Rabbits	--	10[1]	2.5[1]
	Rats	1750	50	7.0[2]
Cineb	Rabbits	600 (LD_{100})		
	Rats	4775 (industrial)		10
Maneb		5270 (pure)		
	Mice	4000 (industrial)		
Cupracin 1	Rats	4080±682 (industrial)	40	7[2]
Marcine	Rats	1216±28.8 (industrial)	--	--
Polycarbacine	Rats	6100±1.300	61	

[1]Data of N. G. Fel'dman, R. A. Ryazanova, V. Kh. Yenikeyev (1969).

[2]Threshold.

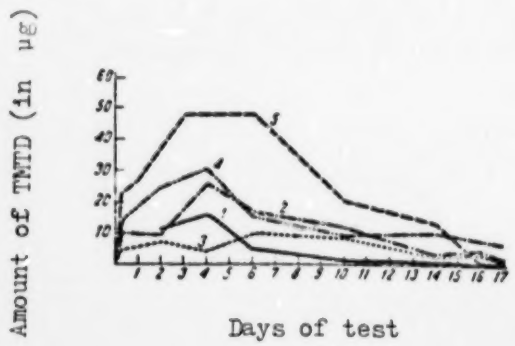

Fig. 1. Dynamics of the amount of TMTD in blood, urine and tissues of rats.

1--urine; 2--lungs; 3--muscles; 4--brain; 5--blood.

killed on the 20th day of pregnancy. We counted the number of yellow bodies and resorbed fetuses, the number of stillborns, characteristics of postnatal development, survival and developmental deformities. All observed forms of pathology were combined under the heading "unfavorable outcome of pregnancy" (Table 2).

TABLE 2. EMBRYOTOXICITY OF DITHIOCARBAMATES

Pesticide	Dose (in mg/kg)	Unfavorable outcome of pregnancy (in %)
Cirame	250	97.8
	42	22.6
	14	4.4
Cineb	300	22.6
	50	11.5
Maneb	50	21.2
	25	25.0
	5	30.0
	1	5.2
Polycarbacine	610	Teratogenic effect
	.61	8.9
Control group of animals	--	12

All these pesticides had an embryotoxic effect, which was expressed in resorption of fetuses, high litter mortality, low weight of newborns and retardation of postnatal development. Ciram, polycarbamacine and maneb caused development anomalies--defects of bones of the skull, shortening and absence of extremities and tail, cleft palate, etc. According to the severity of the embryotoxic effect, on the basis of the results of our studies and the data of R. Y. Ryazanova, pesticides can be arranged in the following descending order: maneb, cirame, cineb, polycarbacine; in order of teratogenic effect--cirame, maneb, polycarbacine.

Gonatodotic dithiocarbamates were studied by administering them in a dose of 2 mg/kg to sexually immature male and female rats for 50 days and their subsequent pairing among themselves and with intact males and females. The character of the estrous cycle was studied in females and the functional state of spermatozoons in males. It was established that gonadotoxicity of these dithiocarbamates is more pronounced in cirame and least in cineb. Polycarbacine, even in a dose of 610 mg/kg, had no gonadotoxic effect.

The blastomogenic activity of TMTD, cirame, cineb and maneb was studied in black mice of the low-cancer C57 strain and random bred white mice. The animals were given the pesticides once a week in the stomach, suspended in starch paste. As was shown, all pesticides have a weak blastomogenic activity (Table 3).

A study of the dynamics of pathological-morphological and histochemical processes of blastomogenesis showed that in 2-6 weeks after the end of administration of the poison, diffuse thickening of alveolar walls was already observed, because of the proliferation of alveolar and histiocytic cellular elements, the appearance of atypical large cells with light cytoplasm and hyper- or hypochromic nuclei. By the end of 3 months adenomas with a tubular structure developed. By 6 months adenomas were more mature and had, as a rule, a papillary structure. Proliferation of bronchial epithelium was also noted.

From the 2nd week the amount of RNA in bronchial epithelium and the number of atypical cells gradually increased, the activity of oxidation-reduction enzymes increased.

Blastomogenetic processes under the influence of cineb, cirame and maneb are qualitatively very similar to those which were observed with the administration of urethan. With prolonged (9 months) administration of pesticides with food in a dose of 2 mg/kg no mature tumors appeared in black C57 mice. However, morphological changes developed in the lungs which can be considered pre-cancerous. The most pronounced blastomogenic effect was observed with maneb and the least with TMTD.

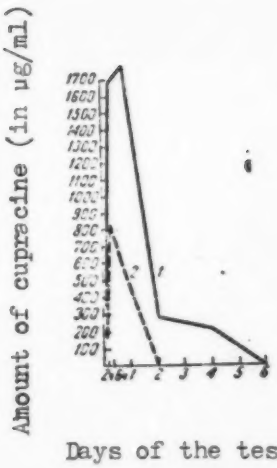

Fig. 2. Dynamics of the amount of cupracine in adrenals and thyroid gland of rats.

1--adrenals; 2--thyroid gland.

TABLE 3. BLASTOMOGENIC ACTIVITY OF DITHIOCARBAMATES

Pesticide	Kind of animals	Dose (in mg/kg)	Number of administrations	Observation time after ending administrations (in months)	Percent of animals with tumors	
					test	control
	Mice, white, random bred	1300	6	7	30.0	8
Cineb	Mice, C57 black	1750	11	6	7.6	0
	Mice, C57 black	75	20	6	7.4	0
Cirame	Mice, white random bred	60	14	7	40.0	10.0
Maneb	Mice, C57 black	600	6	9	9.5	0
TMTD	Mice, C57 black	300	6	6	4.0	0

We studied the effect of cineb, cirame, maneb and TMTD on chromosomes of human and animal somatic cells in tests in vitro and in vivo. In treating a lymphocyte culture of whole human blood with cirame in doses of 0.06, 0.015 and 0.003 µg/ml, the number of cells with damaged chromosomes was statistically reliable, it exceeded that in the control group and was 13.33, 9 and 5.5% respectively. With a dose of 0.0006 µg/ml the difference in the number of aberrant cells between the test and control cultures was statistically unreliable. The preparation induced both chromatid and chromosome type aberrations in a ratio of 2.7:1.

To determine the cytogenetic activity of cirame we studied the frequency of chromosome aberrations in lymphocytes of peripheral blood in workers who came in contact with this substance in industry. The number of metaphases with aberrations in the group examined was almost 6 times higher than that in the control, 5.9%. An increased number (in comparison with the control) of both chromatid and chromosome type aberrations was found (in a ratio of 2.3:1). According to basic quantitative indices of the cytogenetic effect, the results obtained in treating cultures with cirame in a concentration of 0.003 µg/ml corresponds closely to data in vivo.

Maneb in doses of 0.5, 15 and 30 µg/ml in a lymphocyte culture of whole human blood increased the frequency of chromosome aberrations to 12-22% (2% in the control). No dose dependence was found in the limits of these concentrations. When exposure was increased to 48 hours, the number of aberrations, with the same dose of maneb (15 µg/ml), increased to 55%.

In tests in vivo on rabbits, maneb also revealed cytogenetic activity (A. V. Samosh). With a single administration of the preparation to the stomach in a dose of 800 mg/kg, chromosome aberrations reliably increased their frequency more than 7 times in 24 hours in bone marrow cells of the test animals in comparison with the control (7.8 and 1%). It was maintained for 48 hours after the effect (8.8%). When rabbits were given a single inoculation of maneb in a dose of 14 mg/kg, with the same fixation time, the level of chromosome

aberrations did not differ from that in the control (1.5 and 1%). However, with multiple peroral administration of maneb in the very same dose (14 mg/kg) for 4½ months, the frequency of chromosome damage increased more than 5 times in comparison with the control (5.8 and 1%). These results indicate that prolonged contact with a small dose of the preparation, having no effect with a single exposure, can present a cytogenetic danger for somatic bone marrow cells. According to the type of chromosome aberrations, maneb induced primarily chromatid breaks with the formation of fragments both in vitro in human cells and in a rabbit. Characteristics of chromosome damage found in human lymphocytes in culture correspond to those in bone marrow cells of the rabbit.

In administering TMTD to mice in doses of 20, 100 and 1000 mg/kg, the number of cells with chromosome damage after 24 hours was 1.14, 3.9 and 7.83% respectively. In 48 hours after the administration of TMTD in a dose of 1000 mg/kg the number of metaphases with fragments was reduced to 3.7%. Taking into consideration that with peroral administration part of TMTD disintegrates in the stomach, we determined the activity of the preparation when it is administered intraperitoneally in a dose of 100 mg/kg. 9.5% of metaphases were found with damaged chromosomes, i.e. in 2½ times more than with administration to the stomach of the same dosage of the substance.

A comparative evaluation was made of the effect of TMTD, cirame, cineb and maneb on the chromosomes of bone marrow cells of mice with peroral administration in a dose of 100 mg/kg. In the bone marrow of mice, subjected to cirame, cineb and maneb, 3.13, 1.4 and 1.8% of cells with chromosome damage were found. As was indicated above, under the effect of TMTD in a dose of 100 mg/kg, the number of metaphases with aberrations was 3.9%. Thus, in ability to cause chromosome damage in the bone marrow cells of mice, TMTD stands first, then follow cirame, maneb and cineb (Table 4).

Dithiocarbamates and their conversion products were determined by the colorimetric group method according to carbon bisulfide and by means of thin layer chromatography. We did not intend to give a detailed hygienic assessment

TABLE 4. FREQUENCY OF CHROMOSOME DAMAGE IN BONE MARROW CELLS OF MICE WITH A SINGLE EFFECT OF DITHIOCARBAMIC ACID DERIVATIVES IN A DOSE OF 100 mg/kg

Substance	Fraction time (in hours)	Number of animals	Number of metaphases counted	Number of metaphases with aberrations	
				Absolute	%
TMTD	24	7	640	25	3.90±0.761
Control I	--	12	1100	8	0.73±0.25
Cirame	24	6	510	16	3.13±0.768
Maneb	24	6	500	9	1.80±0.60
Cineb	24	6	500	7	1.40±0.525
Control II	--	7	650	3	0.46±0.264

of food products treated with dithiocarbamates or suggest conditions for their use, but we feel it is necessary to turn the attention of hygienists and toxicologists to several facts of primary importance. As a result of the study it was established that under natural conditions dithiocarbamates are destroyed in plants as well as in water, forming a number of compounds also detectable in the organism of poisoned warm blooded animals (ethylenethiourea, tetramethylthiourea, dimethylamine salt of dimethyl dithiocarbamic acid, elementary sulfur). It was also noted that the rate of the breakdown of dithiocarbamates in different crops and different parts of the same crop, as well as the character of conversion, is dissimilar.

Thus, in black currants, treated with cirame, tetramethylthiourea and dimethylamine salt of dimethyldithiocarbamic acid predominate, and in apples--TMTD. In the leaves of sugar beets treated with cineb and polycarbacine and apples treated with maneb, ethylene thiourammonosulfide predominates, and in strawberries sprayed with polycarbacine--ethylenethiourea. We must also point out that under certain conditions of using cineb, polycarbacine and cupocine 1 (number of treatments of the plants, kind of crop, length of time waited) only their conversion products are noted in the plants.

In summary we must note that dithiocarbamates belong to the group of pesticides considered dangerous to human health. Several (cirame, TMTD) are highly toxic, have a very pronounced cumulative effect and can cause chronic poisoning with daily administration to the stomach in small amounts. However, the basic indices of the danger of dithiocarbamates are not their toxic properties, but their so-called side effects. All these disturb embryogenesis, and have cytogenetic and blastomogenic activity.

The degree all effects are expressed in different poisons varies, which also is responsible for their differing degrees of potential danger. Comparatively evaluating TMTD, ciram, cineb and polycarbacine, taking into account all their possible pathological effects, it must be pointed out that the most dangerous are ciram, TMTD and maneb. The pathological effects we studied are least pronounced in cineb and polycarbacine, which leads us to consider these substances less dangerous to man than other pesticides in this class.

In connection with the fact that dithiocarbamates are subject to destruction in food crops and water and that it is possible that such compounds as ethylenethiourea, ethylene thiourammonosulfide and tetramethylthiourea can develop in the human organism with nutrition products, it is very important from the hygiene point of view to study the biological effect of these compounds and in particular to find out if the so-called side effects of dithiocarbamates are caused by the original compounds or their conversion products.

REFERENCES

1. Antonovich Ye. A., Vraginskiy S. Ye. Tezisy dokl. k plenumu Goskomissii po toksikologicheskoy i gigienicheskoy otsenke novykh pesticidov, prokhodivshikh ispytaniya v 1967. (Thesis of report to plenary session of State Commission on Toxicological and Hygienic Assessment of New Pesticides, tested in 1967). Moscow, 1968, p. 116.

2. Antonovich Ye. A., Vekshteyn M. Sh. Tezisy dokl. k plenumu Goskomissii po toksikologicheskoy i gigienicheskoy otsenke novykh pestitsidov, prokhodivshikh ispytaniya v 1968-1969. (Thesis of report to plenary session of State Commission on Toxicological and Hygienic Assessment of new pesticides tested in 1968-1969). Moscow, 1970, p. 27.

3. Ryazanova R. A. Gig. i san. 1967, No. 2, p. 26.

4. Fel'dman N. G., Ryazanova R. A., Yenikeyev V. Kh. In the book: Gigiena primeneniya, toksikologiya pestitsidov i klinika otravleniy. (The Hygiene of use, toxicology of pesticides and clinical characteristics of poisoning). Kiev, 1969, p. 290.